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There remains of course the problem of identifying the free activator in the medium of the sperm suspension by its only known mode of operation, that of fertilizing the ovum. This problem, over which several investigators have broken their weapons, appears in a somewhat different light as a result of these experiments; and new experiments should therefore be undertaken.

VARIATION IN BACTERIA

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The bearing of slight physiological differences upon the classification of bacteria and upon the phenomena of infection has made the occurrence of variation among bacteria fully as conspicuous as in the higher forms of life. During the past ten years many observations have been recorded upon the extent and nature of bacterial variability. In these studies some confusion has arisen through the difficulty of distinguishing true variations from the development of latent characteristics, and from environmental modification.

By the term 'latent characteristics' is meant those qualities or properties that are dormant in the organism or cell and are manifested only in response to definite external influences. Thus, certain bacteria form spores in the presence, but not in the absence, of oxygen; some bacteria are known that develop conspicuous capsules when growing in the animal body, but lack these envelopes in part or altogether in artificial media; according to Wright, animal fluids seem to be essential for the production of the characteristic clubs of *Actinomyces* colonies. The sudden appearance in this way of a definite morphological character cannot be looked upon as an instance of variation. Such a manifestation is merely an immediate response to changed conditions of life and is of exactly the same kind as the marked difference in the aquatic and terrestrial forms of *Polygonum amphibium* referred to by DeVries, or as shown in the transformation of the shrimp *Artemia salina* into what some writers consider another species, *Artemia milhausenii*, when the former is transferred to water of a greater degree of saltiness. In no sense is the awakening of such a dormant character to be confounded with true variation. In other words the power to produce certain structures or certain physiological effects exists ready formed in the specific

bacterial cell and is evoked by proper stimuli. These latent qualities often play an important part in bacterial identification, and it is frequently impossible to recognize a given organism until it is brought into a particular environment and has given the appropriate response. Bacteria are very sensitive to slight chemical differences in their surroundings and many alleged instances of variability in bacteria are simply differences in response evoked by variations in the composition of culture media. Herein lies the justification for the attempts at standardization of culture media and the maintenance of exact uniformity in the conditions selected for bacterial growth. The true latent character appears promptly and typically when the suitable conditions for its manifestation are afforded, and not under other circumstances. I have in my laboratory a culture of a bacillus discovered by one of my students (M. Didlake, *Centralbl. Bakt.* II, 15, 193, 1905) that gives a brilliant red pigment in agar prepared from the soy bean, but fails to produce the pigment upon the ordinary meat broth peptone agar or upon any other medium tested. This is a clear case of a latent characteristic and is to be ranked with the fermentation of rare carbohydrates—as rhamnose by the paratyphoid bacillus—by microorganisms that in nature rarely if ever come in contact with the substances that they unhesitatingly attack.

What are called *environmental modifications* are sometimes hardly to be distinguished fundamentally from the phenomenon just under consideration. In a sense these modifications depend upon the possession of an innate capability for response to a definite environment, and as such must be regarded as expressions of latent characteristics.

Such modifications in the higher forms of life are typically the effects of use and disuse, of a more or less abundant food-supply or of climatic factors. One important difference between such modifications and the bursting into bloom of latent characteristics is that environmental influences of the nature indicated tend to produce similar results in different organisms. Among bacteria in general, latent characteristics are more susceptible of demonstration than environmental modifications. It is often difficult in practice to distinguish genuine environmental modifications from adaptations due to selection, although the phenomena are totally distinct. If one-half of an alpine plant be set in a low altitude garden, the other half being left in its original habitat, differences will arise which may be reasonably ascribed to environmental modification. With bacteria, experimentation of this sort is not so simple. The enormous number of generations through which a given culture of bacteria can pass in

a short time may convey superficially the impression of an environmental modification similar to that observed in an individual organism transplanted into different surroundings, when in reality there has been simply elimination of unsuited variations and a selection of the variety adapted to the particular conditions. This is a serious obstacle in the way of obtaining a proper appreciation of direct environmental influence upon the individual bacterial cell.

The great majority of bacterial variations that have been described belong to the class of apparently adaptive modifications, whether such modification be considered as due to the direct action of the environment upon thousands or millions of bacterial cells, or to the superior advantage in the intra-cultural competition possessed by those cells that have some new peculiarity such, for instance, as that of fermenting a particular carbohydrate. A bacterial culture, brought in contact with a carbohydrate that it is unable to attack, may undergo a change so that it becomes able to split the carbohydrate, the change being then apparently adaptive. Such cases have been frequently cited as instances of bacterial mutation, but, as many of them are described, they might equally well be regarded as due to selective acting upon the so-called fluctuating variations. It is worth noting that most or all the cells of certain species seem to behave in the same way in the presence of certain fermentable substances, and that the newly acquired property is sometimes permanent for a long series of generations on other media, sometimes lost.

In the course of some experiments I have been carrying on during the past three years I have attempted to determine the width of swing in a pure line strain of bacteria, *B. coli*, cultivated under varying conditions. From a freshly cultivated feces culture a single cell was isolated by the Barber method, and from the descendants of this cell, numbering some hundreds of millions, two other cells taken at random were made the parents of two strains which have been used in a long series of experiments. Without space left to consider the technical details the following results may be stated. Both strains have been subjected to a series of influences, some of which might be expected to lead to particular adaptive modification, others of which were of a less specific character. Neither strain in the course of growth for over 500 generations at 37° C. (transfer every two days) on ordinary nutrient agar has shown any permanent change in ability to produce indol, to coagulate milk, or to ferment carbohydrates. On several occasions, however, variations in indol production were observed (four days at 37° C.—Ehrlich method)

so that the results as recorded range from a mere trace to the maximum intensity observed in any culture. The tests were always carried out on the same lot of culture medium and were controlled by a number of cultures (usually about 20) from the same strains but of different cultural histories. Milk was always rendered acid but sometimes not coagulated in forty-eight hours. The milk used was certified milk always obtained from one dealer and treated in as uniform a manner as possible throughout. Nevertheless, differences in rapidity and completeness of coagulation were so great in different lots of milk as to make such changes of little value in attempts to study variation in the physiological properties of the organisms themselves.

The most fundamental change thus far observed is the acquisition of saccharose-fermenting powers by one of the pure line strains. This quality appeared in the seventh transfer on sodium chloride agar. It was not manifested by all the cells of the culture, but at the time of examination the saccharose-fermenting cells were greatly in the majority. On continuing the transfers they became the sole type found in the cultures, the non-saccharose type disappearing altogether. The power of fermenting raffinose was also possessed by the saccharose-fermenting strains. Gas is produced in both saccharose and raffinose solutions and over 4% of normal acid is formed. The newly acquired fermenting property has remained permanent throughout a series of over 500 test-tube generations (forty-eight-hour transfers), and is shown both by the cultures on sodium chloride media and by the strains transferred immediately on the acquisition of this property to ordinary nutrient agar and grown side by side with the two parent strains.

This instance of bacterial mutation therefore seems to fulfil the requirements (*a*) of appearing suddenly without intermediate stages, (*b*) of being irreversible, at least for three years and for some hundreds of test-tube generations, (*c*) of comprising change in two characters (saccharose and raffinose-fermenting power), and (*d*) of not involving all the cells of the parent strain.

It may be remarked that a differentiation of *B. coli* into species is commonly made on the basis of the power to ferment saccharose and most of the recent classifications of *B. coli* groups start with this as a fundamental distinction. In the great majority of strains of *B. coli* that have been tested by various observers saccharose fermentation is correlated with raffinose fermentation. Saccharose-fermenting streptococci on the other hand are often devoid of power to ferment raffinose.

It may be noted further that the acquisition of this new character by an originally non-saccharose-fermenting strain of *B. coli* has thus far developed only once and then on sodium chloride medium. Cultures of the parent organism grown in saccharose broth for a series of generations as yet show no gas production or acid production. This particular change therefore seems to be due to the intra-cellular or molecular changes brought about by non-specific influences and not to a direct adaptation to particular environmental conditions.

A DYNAMIC CONCEPTION OF THE ORGANIC INDIVIDUAL

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The organic world exists in the form of more or less clearly defined individuals, which may be completely isolated from others as self-maintaining organisms, or only partly isolated, like the members of a so-called colony among the lower animals and the different buds or growing tips and the parts associated with each in the multi-axial plants. A tree, for example, consists of a great number of plant individuals organically connected with each other.

In all except the very simplest organic individuals an orderly, definite sequence of events in space and time occurs which we call development. Development includes the series of changes from the reproductive cell or cell mass to the mature form of the organism. On the one hand, the organs arise in definite space relations to each other and to certain axes or planes which we can draw through the developing organism, and on the other hand, development consists in an orderly sequence of events in time. Certain regions always precede and others follow in regular order. The result of this orderly behavior is an organism of more or less definite form and structure, often exceedingly complex and with a high degree of constancy in successive generations. In the simplest individuals these space and time sequences are either less definite or else they are subject to frequent change and replacement by others, but in most organisms they are relatively permanent.

Most theories of the organism have failed to account satisfactorily for these orderly characteristics. Either they have simply assumed the existence of some sort of mechanism adequate to account for the facts, or some 'vitalistic,' i.e., non-mechanistic principle, as a controlling or